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DOI: 10.1016/j.micpath.2013.12.006 · Source: PubMed

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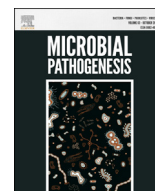
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## Virulence factors of *Helicobacter pylori* *vacA* increase markedly gastric mucosal TGF- $\beta$ 1 mRNA expression in gastritis patients



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### ARTICLE INFO

#### Article history:

Received 8 September 2013

Received in revised form

14 December 2013

Accepted 30 December 2013

Available online 21 January 2014

#### Keywords:

*Helicobacter pylori*

Gastritis

TGF- $\beta$ 1

Virulence factors

### ABSTRACT

**Objective:** *Helicobacter pylori* (*H. pylori*) infection is the main cause of gastric inflammation. Regulatory T cells (Treg cells) suppress the activation and proliferation of antigen-specific T cells and mediate immunologic tolerance. TGF- $\beta$ 1 was shown to be secreted in a subset of Treg cells known as 'Th3 cells'. These cells have not been sufficiently studied in context to *H. pylori*-induced inflammation in human gastric mucosa. In this study we therefore, aimed to investigate the expression of TGF- $\beta$ 1 in the context of *H. pylori* colonization in chronic gastritis, to examine the relationship between it and histopathologic findings and to compare it with virulence factors.

**Patients and methods:** Total RNA was extracted from gastric biopsies of 48 *H. pylori*-infected patients and 38 *H. pylori*-negative patients with gastritis. Mucosal TGF- $\beta$ 1 mRNA expression in *H. pylori*-infected and uninfected gastric biopsies was determined by real-time PCR. Presence of *vacA*, *cagA*, *iceA*, *babA2* and *oipA* virulence factors was evaluated using PCR.

**Results:** TGF- $\beta$ 1 mRNA expression was significantly increased in biopsies of *H. pylori*-infected patients compared to *H. pylori*-uninfected patients. There was association between virulence factors and TGF- $\beta$ 1 mRNA expression. TGF- $\beta$ 1 mRNA expression in mucosa was significantly higher in patients with *vacA* s1 and s1m1.

**Conclusions:** TGF- $\beta$ 1 may play an important role in the inflammatory response and promote the chronic and persistent inflammatory changes in the gastric. This may ultimately influence the outcome of *H. pylori*-associated diseases that arise within the context of gastritis and *vacA* may suffice to induce expression of TGF- $\beta$ 1 mRNA.

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### 1. Introduction

Colonization by *H. pylori* is known to cause chronic gastritis and lead to the development of severe gastroduodenal diseases such as peptic ulcers, lymphoma of the mucosa associated lymphoid tissue (MALT) or gastric adenocarcinoma [1]. Activation and migration of these inflammatory cells into the gastric mucosa is related to increased production of pro-inflammatory cytokines, including IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\beta$ , IL-18, IL-17, IL-8, IL-6 and various cytokines

which are believed to contribute to maintaining the gastric inflammation and causing epithelial cell damage [2–5]. The risk of different clinical expression of *H. pylori*-infection is thought to rely on interactions between the host genetic factors and bacterial factors. For instance, polymorphisms of pro-inflammatory cytokine genes such as IL-8, IL-10, IL-17, IL-1 $\beta$  and TNF- $\alpha$  that enhance inflammatory response of gastric mucosa, have been correlated to an increased risk of gastric cancer and peptic ulcer [6–11]. It has been shown that the mucosal levels of several cytokines are significantly higher in *H. pylori*-infected with virulence factors groups in comparison to *H. pylori*-infected without virulence factors groups [12–14]. Even though mucosal *H. pylori* colonization induces vigorous immune responses involving both the innate and adaptive immune system, the infection is not removed, and a state of chronic active

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gastritis persists for life if untreated [15]. During *H. pylori* infection, T cells are generally hyporesponsive and infected gastric tissues have also shown the presence of transforming growth factor  $\beta$  (TGF- $\beta$ ), which has a suppressive effect on T cells [16–18]. Recent studies have demonstrated that CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells (Tregs) suppress the immune response against *H. pylori* [19–21]. Mouse model studies have shown that *in vivo* depletion of Tregs in infected mice leads to increased gastric inflammation and reduced colonization of *H. pylori* [20]. The dysfunction or depletion of Treg cells is associated with autoimmune diseases, allergy and inflammatory bowel disease [22]. Today, it is established that regulatory T cells include two distinct subsets, the naturally occurring FOXP3-expressing CD4<sup>+</sup>CD25<sup>high</sup> Treg cells, Tr1 cells secreting interleukin IL-10, and Th3 cells characterized by transforming growth factor (TGF- $\beta$ 1) secretion [23]. Among the FOXP3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup> Treg cells, two distinct subsets can be distinguished: (a) thymus derived naturally occurring FOXP3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup> Treg cells and (b) peripherally induced FOXP3<sup>+</sup>CD4<sup>+</sup>CD25<sup>high</sup> Treg cells. For the differentiation of the latter subset, TGF- $\beta$ 1 has been reported to play an important regulatory role by induction of FOXP3 gene expression in naive CD4<sup>+</sup> T cells at the site of inflammation [24]. In animal models, the functional role of Treg cells in the context of *H. pylori*-infection has been investigated, recently. During interaction with *H. pylori*, FOXP3 expressing Treg cells suppressed the immune response toward the bacterium [25]. Gastric Treg cells were able to actively suppress *H. pylori*-induced T-cell proliferation and INF- $\gamma$  production [19]. Moreover, *H. pylori*-infection is an established risk factor for gastric cancer. In fact, the population of Tregs in tumor-infiltrating lymphocytes is significantly larger than in normal tissue in several malignancies, including gastric cancer [19,26–29]. These findings suggest that Tregs contribute to the persistence of *H. pylori*-infection, which may be closely related to gastric carcinogenesis. Regulatory T cells are thought to be a functionally unique subset of CD4<sup>+</sup> T cell populations and function to maintain immune homeostasis [30,31]. The aim of this study was to investigate the expression of TGF- $\beta$ 1 in the context of *H. pylori* colonization in chronic gastritis, to examine the relationship between it and histopathologic findings and to compare it with virulence factors.

## 2. Materials and methods

### 2.1. Patients and sampling

In this retrospective study a total of 86 subjects, undergoing endoscopy at Hajar University Hospital, were included (Table 1). The study was approved by the human research ethics committee at Shahrekord University and informed consent was obtained from each volunteer before participation. The groups were characterized as follows: the *H. pylori*–positive group ( $n = 48$ : 16 males, 32 females; mean age:  $42.4 \pm 14.82$  years); the *H. pylori*–negative group ( $n = 38$ : 18 males, 20 females; mean age:  $38.18 \pm 16.51$  years). *H. pylori*-infection was determined by the rapid urease test, PCR (16sRNA, glmM) and histological examination of biopsies taken from the corpus. Patients were classified as *H. pylori*-infected only if the four tests were positive, respectively. Four biopsies were collected from 86 *H. pylori*-infected, and used for rapid urease test, histological examination, assessment of bacterial virulence factors, detection of *H. pylori* and cytokine RNA analysis.

### 2.2. Histological examination

Sections of biopsy specimens were embedded in 10% buffered formalin and stained with hematoxylin and eosin to examine gastritis and with giemsa to detect *H. pylori*. The histological

severity of gastritis was blindly graded from normal to severe based on the degree of mononuclear cell (MNC) and polymorphonuclear leukocyte (PMN) infiltration, and atrophy according to the Updated Sydney system [32] on a four-point scale: 0, no; 1, mild; 2, moderate; and 3, severe changes.

### 2.3. PCR amplification

DNA for polymerase chain reaction (PCR) was extracted using the biospin tissue genomic DNA extraction kit (BioFlux, Japan). Specific primers for PCR amplification of different genes are shown in Table 1. For vacA, cagA, iceA1, iceA2 and babA2 evaluation, the PCR program comprised 35 cycles of denaturation (at 94 °C for 30 s), annealing (at 56 °C for 30 s, extension at 72 °C for 30 s), and one final extension (at 72 °C for 5 min). For oipA, amplification was performed with 35 cycles of denaturation (at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s), and one final extension (at 72 °C for 5 min).

### 2.4. Quantitative analysis for TGF- $\beta$ 1 mRNA in the gastric mucosa using real-time PCR

Total RNA was isolated from whole gastric biopsy specimens using total RNA extraction biozol (bioflux, Japan). An aliquot containing 0.2  $\mu$ g of total RNA was used for the reverse transcription reaction, which was conducted using the superscript first-strand cDNA synthesis system (Fermentas, Finland), according to the manufacturer's instructions. The sequences of oligonucleotide primer and probe are shown in Table 2. The quantification of TGF- $\beta$ 1 mRNA levels was performed using a Rotor-Gene 3000 (Corbett, Australia). Real-time PCR reactions were performed in a total volume of 25  $\mu$ l containing 3  $\mu$ l of synthesized cDNA solution, 12.5  $\mu$ l of 2 $\times$  Rotor-Gene Probe PCR Master Mix (Qiagen, Germany), 500 nM of each primer and 250 nM of the TaqMan probe. Amplification program included a pre-warming step (10 min at 94 °C), denaturation step (94 °C for 15 s) and an annealing/extension step (60 °C for 60 s). The expression levels of cytokine mRNA were expressed as the ratio of cytokine mRNA to  $\beta$ -actin mRNA (cytokine mRNA/ $\beta$ -actin mRNA). Each assay was performed in duplicate and each cytokine assay was performed testing all RNA samples in the same experiment. Relative quantification of cytokine to  $\beta$ -actin (cytokine mRNA/ $\beta$ -actin mRNA) was determined using  $2^{-\Delta\Delta Ct} = 2^{-(Ct, \text{ TGF-}\beta\text{1} - Ct, \beta\text{-actin})}$  method [39].

**Table 1**  
PCR primers for amplification of virulence factors.

Primer designation	Primer sequence	Size of PCR product (bp)	References
vacA m1/ m2	vacAmF: 5'-CAATCTGTCCAATCAA GCGAG-3'	567 bp (m1)	[33]
	vacAmR: 5'-GCGTCTAATAATTC C AAGG-3'	642 bp (m2)	
vacA s1/ s2	VA1-F: 5'-ATGGAATACAACAAA CACAC-3'	259 bp (s1)	[34]
	VA1-R: 5'-CTGCTTGAATGCGCAAAAC-3'	286 bp (s2)	
oipA	oipA-F: CAAGCGCTTAACAGATAGGC-3	430 bp	[35]
	oipA-R: AAGCGCTTTCTGCTGAAG-3		
iceA1	iceA1F- 5-GTGTTTTTAACCAAAGTATC-3	247 bp	[36]
	iceA1R-5-CTATAGCCAATCTTTGCA-3		
iceA2	iceA2F: 5-GTTGGGTATATCACAAT TTAT-3	229 or 334 bp	[36]
	iceA2R: 5-TTRCCCTATTTCTAGTA GGT-3		
babA2	bab7-F: 5'-CCAAACGAACAAAAA GCGT-3	271 bp	[37]
	bab7-R: 5'-GCTTGTGTAAAGCCG TCGT-3		
cagA	cag1: 5-ATGACTAACGAAACTATT GATC-3	232 bp	[38]
	cag2 5-CAGGATTTTIGATCGCTTTATT-3		

**Table 2**  
Primer and probe sequences employed in this study.

Gene	Primer and probe sequence
β-actin	Forward 5-AGCCTCGCCTTTGCCGA-3
	Reverse 5-CTGGTGCCTGGGGCG-3
	Probe FAM-CCGCCGCCGTCACACCCGCC-TAMRA
TGF-β1	Forward 5-CAGCAACAATTCCTGGCGATA-3
	Reverse 5-AAGGCGAAAGCCCTCAATTT-3
	Probe FAM-CTGCTGGCACCCAGCGACTCG-TAMRA

### 2.5. Statistical analysis

Data were analyzed using GraphPad Prism 5 Demo (GraphPad Software, San Diego, California, USA). Data are expressed as raw and mean if not stated otherwise. Age was analyzed by unpaired Student's *T*-Test. Normality test was used to determine whether a data set was well-modeled by a normal distribution or not. Cytokine expression was presented as mean and differences between infected and uninfected groups were analyzed using the Student's *T*-Test. Also for gene expression values between *H. pylori*-infected patients and virulence factors, the parametric Student's *T*-Test and for comparison of more than two groups, one-way ANOVA test were used. Categorical data (between virulence factors) are presented as frequencies; comparisons were made using the Chi-square or Fisher exact test if <5 samples were in one group. *P* values <0.05 were considered to be statistically significant.

## 3. Results

### 3.1. Genotype

Table 3 gives an overview of the frequency distribution of the vacA s and m alleles and the cagA status. The vacA s1 and m2 alleles as well as cagA were predominant. Mixed vacA s1/s2 genotypes were found in six patients (12.5%) and mixed vacA m1/m2 genotypes were found in four patients (8.3%). The oipA, babA2, iceA1 and iceA2 genes respectively were found in 97.9%, 85.4%, 60.4% and 41.7% of the *H. pylori* positive biopsies (Table 4).

### 3.2. Correlation of vacA alleles and presence of virulence factors

The cagA gene was detected in 60.4% (29 biopsies) of *H. pylori* positive specimens. The association between vacA alleles and presence of virulence factors is described in Table 5. Of the 29 *H. pylori* positive specimens that were positive for cagA, 26 were

**Table 3**  
Frequency distribution of the vacA allele variants s and m and the cagA status for the total of study patients analyzed.

Genotype	Total	
	N	%
<i>vacA</i> s-region		
s1	34	70.8
s2	8	16.7
s1 and s2 <sup>a</sup>	6	12.5
<i>vacA</i> m-region		
m1	13	27.1
m2	31	64.6
m1 and m2 <sup>a</sup>	4	8.3
<i>cagA</i>		
Positive	29	60.4
Negative	19	39.6
Total	48	100%

<sup>a</sup> The mixed genotypes of virulence factors were excluded in quantitative analysis.

**Table 4**  
Frequency of virulence factors in *H. pylori* positive biopsies.

Genotype				Number (%)
oipA	iceA1	iceA2	babA	
+	+	+	+	5 (10.4)
+	+	–	+	20 (41.1)
+	+	–	–	4 (8.3)
+	–	+	+	14 (29.1)
+	–	+	–	1 (2)
+	–	–	+	2 (4.1)
+	–	–	–	1 (2)
–	–	–	–	1 (2)
				48 (100%)

associated with the toxin-producing vacA s1 and only 2 cagA-positive were vacA s2 and 16 of them were associated with the toxin-producing vacA s1m2 and only 7 were vacA s1m1 and 2 strains were vacA s2m2.

### 3.3. TGF-β1 expression is enhanced in *H. pylori*-infected gastric mucosa

TGF-β1 mRNA level was detectable in all samples regardless of whether biopsies were taken from *H. pylori*-infected or uninfected patients. TGF-β1 expression was significantly more in biopsy specimens of *H. pylori*-infected patients compared with uninfected patients (*p* = 0.04) (Fig. 1). If the mean *H. pylori*-positive group is 0.0041 and the mean *H. pylori*-negative group is 0.0004 then the difference in the TGF-β1 mRNA expression in the *H. pylori*-positive compared with the *H. pylori*-negative patients is 0.0041/0.0004 or 8.9-fold.

### 3.4. Effect of vacA allele variants in *H. pylori*-infected on the mucosal TGF-β1 mRNA level in gastric mucosa

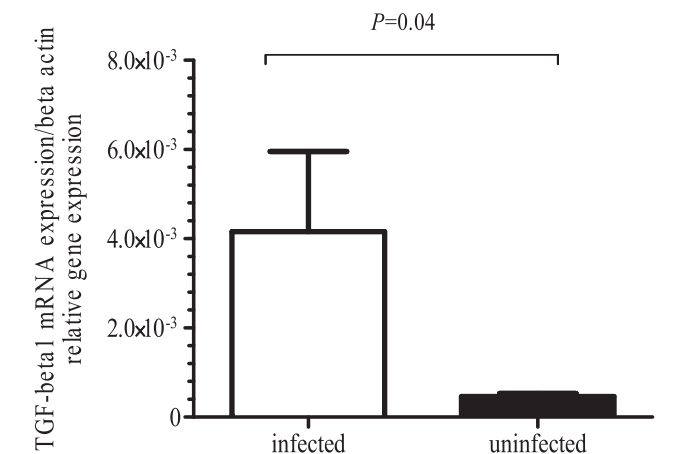
The results showed that in *H. pylori*-infected patients; mucosal TGF-β1 mRNA level was dependent on the vacA status. The difference in the TGF-β1 mRNA expression in the vacA s1-positive *H. pylori* strains compared with the vacA s2-positive *H. pylori* strains is 0.0045/0.0004 or 10.85-fold. Mucosal TGF-β1 mRNA expression in gastritis patients with vacA m1-positive was not significantly higher than those observed in gastritis patients with vacA m2-positive (0.0081/0.0017 or 4.64-fold). Mucosal TGF-β1 mRNA expression in gastritis patients with vacA s1m1-positive was also significantly higher than those observed in gastritis patients with vacA s1m2-positive (0.0081/0.0026 or 3.11-fold) and vacA s2m2-positive (0.0081/0.0004 or 20.25-fold) (Fig. 2).

### 3.5. Effect of others virulence factors in *H. pylori*-infected on the mucosal TGF-β1 mRNA level in gastric mucosa

A mucosal TGF-β1 mRNA level was independent of virulence factors status. If the mean *H. pylori*-positive group is 0.005 and the mean *H. pylori*-negative group is 0.007 then the difference in the TGF-β1 mRNA expression in the cagA-positive *H. pylori* strains compared with the cagA-negative *H. pylori* strains is 0.005/0.0007 or 6.9-fold, the difference in the TGF-β1 mRNA expression in the babA2-positive *H. pylori* strains compared with the babA2-negative *H. pylori* strains is 0.0038/0.0008 or 4.47-fold, the difference in the TGF-β1 mRNA expression in the iceA1-positive *H. pylori* strains compared with the iceA1-negative HP strains is 0.0052/0.0007 or 6.62-fold and the difference in the TGF-β1 mRNA expression in the iceA2-positive *H. pylori* strains compared with the iceA2-negative *H. pylori* strains is 0.0013/0.0048 or 0.27-fold (Fig. 3).

**Table 5**  
Correlation of *vacA* alleles with the *cagA*, *babA2*, *iceA1* and *iceA2* genotype of the samples studied.

<i>vacA</i> genotype	<i>cagA</i>		<i>babA2</i>		<i>iceA1</i>		<i>iceA2</i>	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
s1m1	7	4	10	1	6	5	4	7
s1m2	16	6	16	6	14	8	8	14
s2m2	2	6	7	1	3	5	4	7
<i>P</i> value	0.06		0.993		0.391		0.524	
s1	26	11	30	7	24	13	12	25
s2	2	6	7	1	3	5	4	4
<i>P</i> value	0.018		0.67		0.157		0.352	
m1	7	4	10	1	6	5	4	7
m2	22	14	29	7	21	15	15	21
<i>P</i> value	0.881		0.429		0.826		0.756	



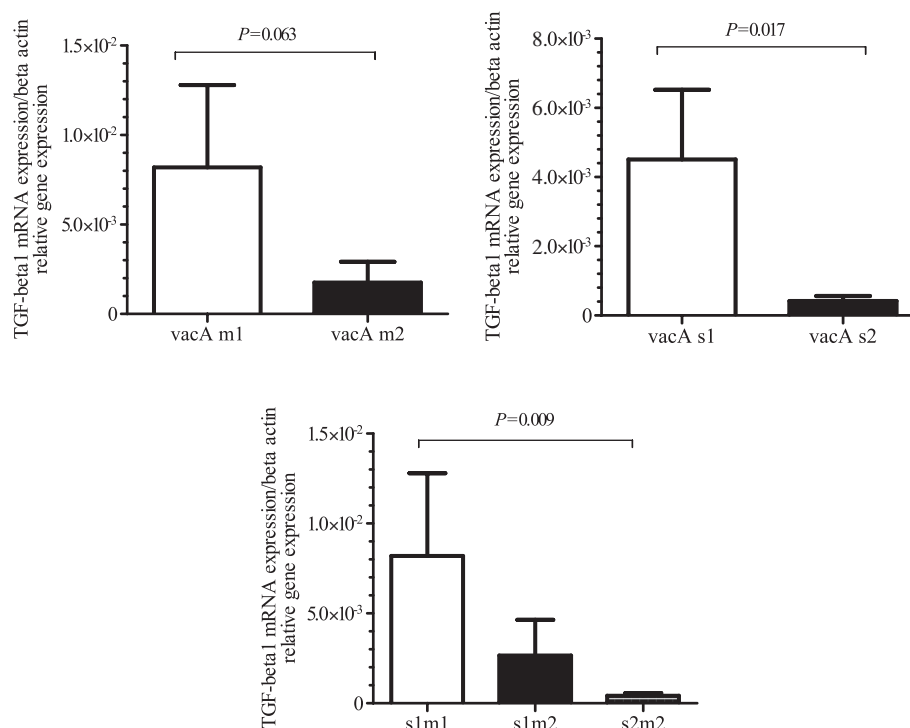
**Fig. 1.** Relative expression level of TGF- $\beta$ 1 mRNA in gastric mucosa shown by *H. pylori* status. RNA was extracted from gastric biopsies of 48 *H. pylori*-infected patients, 38 *H. pylori*-uninfected patients and analyzed for TGF- $\beta$ 1 by real-time PCR. Levels are normalized to  $\beta$ -actin. *P* values <0.05 was considered statistically significant using the Student's *T*-Test.

### 3.6. Correlation between mucosal TGF- $\beta$ 1 mRNA levels and grade of chronic inflammation

The degree of chronic inflammation was assessed and graded as follows: 31.25% [15] mild, 45.83% [22] moderate and 22.9% [11] severe in the studied population. Fig. 4 shows the relationship between the mucosal TGF- $\beta$ 1 mRNA expression and chronic inflammation (mononuclear cell infiltration) scores. There was a significant correlation between the mucosal TGF- $\beta$ 1 mRNA expression and the chronic inflammation scores ( $p = 0.006$ ).

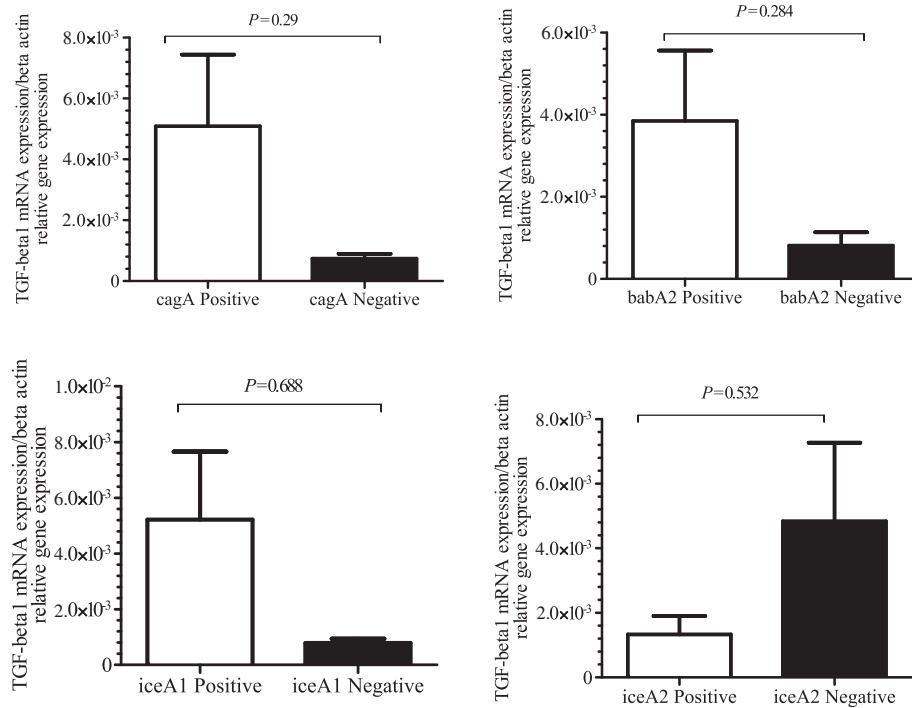
## 4. Discussion

Most *H. pylori* infections begin in childhood and persist lifelong in the host without specific treatment. Although *H. pylori* induce a strong gastric inflammation, the pathogens are not cleared, and chronic or persistent inflammation is generally established. The exact mechanisms by which *H. pylori* escapes the bactericidal effects of gastric acid or how it colonizes the gastric mucosa and damages the gastric epithelial cells by *H. pylori* are not well understood. *H. pylori* induced gastric mucosal damage has been



**Fig. 2.** Relative expression level of TGF- $\beta$ 1 mRNA in *H. pylori*-infected patients shown by *vacA* allelic variants. RNA was extracted from gastric biopsies of 48 *H. pylori*-infected patients (*vacA* s1 = 34 and s2 = 8 patients) (*vacA* m1 = 13 and m2 = 31 patients) (*vacA* s1m1 = 12, s1m2 = 18 and s2m2 = 8 patients).

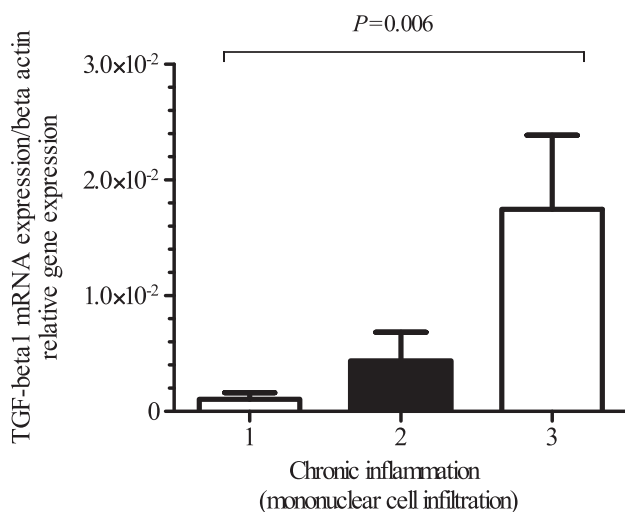




**Fig. 3.** Mucosal TGF-β1 mRNA expression in gastritis patients and virulence factors. RNA was extracted from gastric biopsies of 48H. pylori-infected patients. Levels are normalized to β-actin. P values <0.05 was considered statistically significant using the Student's T-Test.

explained by an imbalance between host defense mechanisms and bacterial cytotoxic factors. The *H. pylori* vacuolating cytotoxin (vacA) recently has been shown to contribute to persistent infection by inhibiting the proliferation and immune response of T cells [40,41]. Unlike acquired immune system, other investigators [42] have reported that vacA may be a driving factor in efficient non-specific immune system. The role of vacA in *H. pylori* infected gastric mucosa remains unclear but our results indicate that in *H. pylori*-infected patients; mucosal TGF-β1 mRNA level is dependent on the vacA status. The results indicate that vacA s1 and s1m1 are associated with increased mucosal TGF-β1 mRNA level. Environmental factors, host genetics, considerable genetic diversity and

pathogenicity between different *H. pylori* strains may contribute to various clinical outcomes [43]. Recent numerous studies have shown that *H. pylori*-induced chronic inflammation could be associated not with a bacterial virulent factor but rather with the host's immunologic tolerance. Treg cells can suppress the proliferation and activation of antigen-specific T cells and they are described as the key regulator of the immune system in the maintenance of immunologic tolerance. Recently, the close correlation between *H. pylori*-infection and immunosuppressive Treg cells in animal and human models has been reported. It has been demonstrated that the depletion of Treg cells in *H. pylori*-infected mice causes an impressive increase in gastric inflammation and a reduction in bacterial colonization [20]. This study has shown that Treg cells suppress Th1-mediated immune response induced by *H. pylori* to contribute to the bacteria's persistent colonization in the gastric mucosa and therefore may play a major role in inducing chronic gastritis. An *in vitro* study found that CD4<sup>+</sup> memory T cells from the peripheral blood in *H. pylori*-infected patients were less responsive to *H. pylori* antigens than were cells in *H. pylori*-uninfected, whereas depletion of FOXP3<sup>+</sup>Treg cells increased the responsiveness of CD4<sup>+</sup> memory T cells [44]. This study indicated that FOXP3<sup>+</sup>Treg cells suppress CD4<sup>+</sup> memory T-cell responses to *H. pylori* in infected patients and reduce induction of T cell-mediated mucosal damage and T cell mediated protection against bacterial colonization. Study by Lundgren and colleagues indicated that frequency of CD4<sup>+</sup>CD25<sup>high</sup> Treg cells in gastric mucosa *H. pylori*-infected adults increased, compared to uninfected, using immunohistochemistry [25]. This was the *in vitro* study to examine the suppressive function of Treg cells in gastric mucosa of *H. pylori*-infected adults. Harris and colleagues evaluated the gastric histopathology and Treg cells in *H. pylori*-infected adults and children [45]. In spite of similar *H. pylori* densities, the intensity of gastritis in children was noticeably less than that in adults. Treg numbers and immune suppressive cytokine, IL-10, TGF-β1, were markedly higher in the mucosal of *H. pylori*-infected children compared to infected



**Fig. 4.** Relative expression level of TGF-β1 mRNA in *H. pylori*-infected patients shown by grade of gastritis.

adults. This results show that in *H. pylori*-infected children, Treg cell responses downregulate the gastric mucosal inflammation. Associations between the maintenance, development, and induction of Treg cells and TGF- $\beta$ 1 have been studied. Chen and colleagues demonstrated that TGF- $\beta$ 1 induced FOXP3 gene expression in naive CD4<sup>+</sup>T cells at the mucosal of *H. pylori*-infected patients, which was essential for the differentiation of CD4<sup>+</sup>CD25<sup>high</sup>FOXP3<sup>+</sup>Treg cells in peripheral blood [46]. These findings introduce FOXP3<sup>+</sup>Treg cells as a major source of TGF- $\beta$ 1 in *H. pylori* gastritis. Sher and Kullberg indicated that Treg cells down regulate Helicobacter hepaticus-induced colonic inflammation in a mouse model of inflammatory bowel disease through the release of TGF- $\beta$ 1 [47]. Both studies showed that the immunosuppressive activity of TGF- $\beta$ 1 was associated with Treg cells. However, the associations between Treg cells and TGF- $\beta$ 1 in *H. pylori*-infection have not yet been comprehensively studied. In the gut of healthy adults, TGF- $\beta$ 1 is an important negative regulator of the Th1 type immune response [48]. TGF- $\beta$ 1 has been reported to be over expressed in the antral of *H. pylori*-infected adults [17]. The results of our study show that *H. pylori*-infection is associated with increased expression of TGF- $\beta$ 1 in children and that FOXP3<sup>+</sup>Treg cells correlate positively with TGF- $\beta$ 1 expression. Our findings also show that the mucosal TGF- $\beta$ 1 mRNA level is increased in the *H. pylori*-infected patients compared with uninfected patients and is correlated positively with vacA, types of disease, and grade of chronic inflammation in patients infected with *H. pylori*. The results indicate that vacA secreted by *H. pylori* may induce the mucosal TGF- $\beta$ 1 mRNA which contributes to persistent infection by inhibiting the proliferation and immune response of T cells. Understanding how *H. pylori* induces Treg cells in patients infected and how Th1 cells appear to finally override the Treg cell influence on *H. pylori*-induced inflammation in adults warrant critical investigation.

## Acknowledgments

This study was financially supported by research deputy of Shahrekord University of Medical Sciences. The authors are grateful to the staffs of Cellular & Molecular Research Center, Shahrekord University of Medical Sciences and the authorities of the endoscopy unit of Shahrekord Hajar Hospital for their valuable helps.

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